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(54) Title: MURRAYA KOENIGII EXTRACTS FOR TREATING ASTHMA

(57) Abstract: The invention relates to a novel composition containing an extract obtained from the plant Murraya koenigii and useful for the treatment or providing relief from acute asthma, and a process for the preparation of a lyophilized extract containing active principles of the plant Murraya koenigii, and a method for the treatment of asthma.

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MURRAYA KOENIGII EXTRACTS FOR TREATING ASTHMA

Technical Field

The present invention relates to use of principles extracted from the plant *Murraya koenigii* for treatment of asthma. This invention provides a process for the extraction of the principles from the plant *Murraya koenigii*. The invention also provides novel compositions useful in the treatment of asthma and methods for the treatment of asthmatic conditions using the said composition.

Background Art

Respiratory diseases such as asthma are reaching epidemic proportions in both the developed and developing world (Nature, Vol. 402, Supplement, and No. 6760, 1999. A special supplement on allergy and asthma). In countries such as Britain and Australia, the respiratory problem translates to 1 in 4 children under age of 14 years having asthma. The disease causes distress and misery in millions often at a time in their lives when they should be most active. Asthma interferes with sleep, intellectual functioning and recreational activities. At one extreme, asthma can be life threatening leading to occurrence of deaths that are avoidable.

Expert panel report has offered guidelines for diagnosis and management of asthma (JAMA Asthma Information Center, www.amaasssm.org/special/asthma/treatmnt/guide /guideline/ gudelin.htm). Control of factors contributing to asthma severity is another avenue for management (JAMA Asthma Information Center, www.amaasssm.org/special /asthma /treatmnt/guide/guidelin/comp2/comp2.htm). Several therapies and medications have also been suggested (JAMA Asthma Information Center, www.amaasssm.org/special/ asthma/treatmnt/guide/guidelin/comp3/medicat/meditoc.htm and www.amaass.org/special asthma/treatmnt/guide/guidelin/comp3/comp3.htm). Immunotherapy is another route for the treatment (JAMA Asthma Information Center, www.amasssm. org/special/asthma/treatmnt/guide/guidelin/comp2/immunoth.htm). Complementary alternative medicine is also being tried (JAMA Asthma Information Center,www.amaasssm.org/special/asthma/treatmnt/guide/guidelin/comp3/medicat/alternat .hmt). However, no satisfactory medication for cure of the disease has been found.

Herein the active factors in Murra*ya koenig*ii was prepared and used under in vitro system to show its usefulness for relief, treatment and cure of asthmatic problem. The plant *Murraya koenig*ii has been studied and reported to have various medicinal values (Chakraborty, M.P Phytochemistry 1997, Oct; Khan B. A, Indian J of Expt. Biol. 1997

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Feb.; Khan B A Indian J of Physiology and Pharmacology 1996, Apr.; Khan BA Plant Foods Hum. Nutr. 1996 Jun).

The leaf of this plant is widely used in various food preparations in India. Further, the plant is ubiquitous. Thus the prior art suggests that the extracts of the plant have several medicinal and other properties which have been widely investigated. This is adequate proof that the extracts of the plant can be safely consumed by humans without any side effects.

The applicants undertook a study on the extracts of this plant to identify the therapeutic use of the principles of this plant. During their study, the applicants discovered the presence of certain active principles in the leaf of *Murraya koenigii* which surprisingly were found to be useful in the treatment and cure of asthma.

Disclosure of the Invention

The main objective of the present invention is to provide a method for the treatment of asthmatic conditions employing active principles extracted from the plant *Murraya koenigii*.

Another object is to provide a process for the isolation of active principles from the plant *Murraya koenigii* and use the same for relief, treatment and cure of asthma.

Yet another object is to provide a simple, fast and inexpensive process to obtain a mixture of active (four) compounds possessing biological activities useful in the treatment of asthma.

Still another object is to provide novel compositions containing active principles extracted from the plant *Murraya koenigii* and useful in the treatment of asthma.

Summary of the invention

Accordingly, the present invention provides a method for the treatment of asthma, employing principles extracted from the plant *Murraya koenigii*. The invention also provides novel compositions useful in the treatment of asthma and methods for the extraction of active principles from the plant *Murraya koenigii*.

Detailed description of the invention

Accordingly, the invention provides a process for preparing an extract from the plant *Murraya koenigii*, useful in the treatment of asthma, said process comprising the steps of pulverising plant parts, extracting the plant pulverized parts with a solvent at

BNSDOCID: <WO 0232440A1>

ambient temperature, concentrating the extract by filtering and evaporating it under reduced pressure and lyophilizing the concentrate to obtain a lyophilized extract containing active principles of the plant *Murraya koenigii*.

In an embodiment, the plant materials are obtained from plant parts of *Murraya koenigii* selected from garden fresh leaves or leaves dried under shade.

In another embodiment, the leaves are pulverized by conventional methods to get homogenized leaves.

In yet another embodiment, the plant materials are extracted with solvents selected from hydrocarbon solvents, chlorinated solvents, ester solvents, ketonic solvents, alcohols, water and buffers. Thus, the solvents may be selected from the group consisting of petroleum ether (BP 40 – 60°C), petroleum ether (60°C - 80°C), benzene, pentane, hexane, chloroform, dichloromethane, carbon tetrachloride, diethyl ether, tetrahydrofuran, dioxane, acetone, cyclopentanone, ethyl acetate, ethyl formate, methanol, ethanol, n-butanol, water and buffers.

In another embodiment, the concentration of the extract is effected by filtering and evaporating the solvents under reduced pressure at a temperature range of 20°C – 80°C preferably at ambient temperature and lyophilizing the concentrate by conventional methods to obtain a mixture of the active principles.

In yet another embodiment, the extract obtained from the plant *Murraya koenigii* comprises active principles which appear as dark colored solids soluble in dimethylsulfoxide.

In another embodiment, the active principles obtained from the plant *Murraya koenigii* are biocompatible and non-toxic in nature. The active principles when subjected to chromatography are found to have R_f values 0.73, 0.60, 0.34 and 0.14 in chloroform and methanol in the ratio 19:1 and R_f values 0.60, 0.38, 0.24 and 0.15 in chloroform. The active principles exhibit four peaks with retention time of 3.37, 3.49, 4.0 and 5.69 methanol is used as solvent at 254nm. The extraction process is carried out for a period ranging from 1 – 120 hrs, preferably between 12 – 16 hrs.

In still another embodiment, plant material is extracted with appropriate solvents such as methanol or water or buffers in a percolator.

In yet another embodiment, the plant extract made by the methanol/appropriate solvent or water is concentrated under reduced pressure to obtain a mixture containing the active principles.

In another embodiment, the concentrate of the plant extract was lyophilized to render the principles substantially free of solvent or water.

In yet another embodiment, the lyophilized solid obtained as the mixture of active factors present in the leaf of *Murraya koenigii* is used for relief, treatment and cure of asthmatic problem.

The method of preparation of the active factors comprises the following steps:

1) collecting the fresh leaves from the local suppliers,

2) drying the leaves under shade to a moderate degree or to take the fresh leaves as the starting material,

3) powdering the dried or homogenizing the fresh leaves,

4) adding the powder or homogenate in a percolator under the bulk of appropriate solvents; choosing hydrocarbon solvents such as petroleum ether (B.P 40-60^{0}C), petroleum ether (B.P 60-80^{0}C), pentane, hexane, benzene etc.; chlorinated solvents like chloroform, dichloromethane, carbon tetrachloride etc.; etheral solvents such as diethyl ether, tetrahydrofuran, dioxane etc.; ketonic solvents such as acetone, cyclopentanone etc.; ester solvents such as ethyl acetate, ethyl formate etc.; all alcohols such as methanol, ethanol, n-butanol etc.; water and buffers, for extraction,

5) extracting the percolated plant material using a percolator for a period ranging from 1 to 12 hours,

6) evaporating the solvent under reduced pressure at a temperature ranging from 20 to 80°C,

7) lyophilizing or drying the material,

8) storing the processed material in a cool and dry place in an air tight container, and

9) evaluating the bioactivity of the material.

Further, the invention also provides pharmaceutical composition useful in the treatment of asthma, said composition comprising an effective amount of extract obtained from the plant *Murraya koenigii* together with, or associated with a pharmaceutically acceptable additive. The additives used in the composition comprise powder or extracts of plants selected from *M. paniculata Linn, H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees, E. hirta,* and *Murraya koenigii.* These additives are present in the range of 80-100 mg of *M. paniculata Linn*, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T. ammi*, 7-13 mg of *S. aromaticum*, 85 - 115 mg of *A.vasica Nees* and 90-110 mg of *E.hirta.*

In an embodiment, the composition comprises :

M. paniculata Linn. Syn. M exotica (KAMINI)	90mg
H. abelmoschus (JOWAN)	50mg
T. ammi (LAVANGA)	50mg
S. aromaticum (BASAK)	10mg
A.vasica Nees (PUSITOA)	100mg
E.hirta	100mg
Murraya koenigii (Suravi Neem)	100mg

Further, the invention provides a method for the treatment of asthma, said method comprising the steps of administering an effective amount of the composition to a subject in need thereof.

In an embodiment, the lyophilized extract obtained from *Murraya Koenigii* is administered alone or along with other conventional additives for the treatment of asthma.

In still another embodiment, the mode of administration is oral for the treatment of mild or acute asthma.

In yet another embodiment, the dosage level of the composition, comprising the extract from the plant *Murraya koenigii* is between 325-600 mg twice daily for the period ranging from 3 to 30 days.

In another embodiment, the dosage level is in between 325-600 mg twice daily for the period ranging from 3 to 15 days for mild asthmatic condition, and 15 – 30 days for acute asthmatic condition.

In still another embodiment, the additives may be powder or extracts of plants *M. paniculata Linn*, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica Nees*, *E.hirta*, and *Murraya koenigii*.

As said above, the additives are present in a range of 80-100 mg of *M. paniculata Linn*, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T. ammi*, 7-13 mg of *S. aromaticum*, 85-115 mg of *A.vasica Nees*, 90-110 mg of *E.hirta*, and 87-105 mg of *Murraya koenigii*. per dose.

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The composition may be formulated in different physical forms suitable for oral or systemic use, although oral use is recommended.

In another embodiment, the additives obtained from the plants *M. paniculata Linn*, *H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees, E. hirta,* and *Murraya koenigii* are administered to impart properties such as antidiahorial, antiseptic, carminative, stimulation, anti-cough, anti- bronchitis and nourishment to the composition.

In another embodiment, the additives are obtained from : *M. paniculata Linn* (bark or root), *H. abelmoschus* from dried flower buds, *T. ammi* from leaves, *S. aromaticum* from whole plant *A.vasica Nees* from root, *E.hirta* from bark, and *Murraya koenigii* from leaves.

In addition, the invention provides an anti-oxidant composition for human beings and animals, said composition comprising a effective amount of extract obtained from the plant *Murraya koenigii* together with or optionally, associated with additives.

In still another embodiment, the additives comprise powder or extracts of plants selected from *M. paniculata Linn, H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees, E hirta,* and *Murraya koenigii.*

In an embodiment, the additives are present in the range of 80-100 mg of *M. paniculate Linn*, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T.ammi*, 7-13 mg of *S. aromaticum*, 85-115 mg of *A.vasica Nees*, 90-110 mg of *E hirta*, and 87-105 mg of *Murraya koenigii* per dose of the anti-oxidant composition.

As said above, the active factor(s) in *Murraya koenigii* are useful for relief, treatment and cure of asthmatic problem, the preparation of which comprises drying, powdering, and extracting the dried leaves of the plant, *Murraya koenigii*, in a percolator at an ambient temperature using appropriate solvents and concentrating the extract under reduced pressure and finally lyophilizing the concentrate to make the active factors.

The following examples are given by way of illustration and therefore should not be construed to limit the scope of the present invention.

Collection of Plant material

The leaves of *M. koenigii* were collected from shrubs in the month of March-August 2000 from different areas of West Bengal, India. A voucher specimen is deposited at the department of Medicinal Chemistry at the Indian Institute of Chemical Biology, 4, Raja S. C. Mallick Road, Calcutta- 700 032, India.

BNSDOCID: <WO 0232440A1>

Extraction of the active principles from *Murraya koenigii*

Example – 1

Fresh leaf containing branches of *Murraya koenigii* were collected, cleaned and washed with water and used as starting material. It was air-dried under shade and drying was continued till it was brittle to make it suitable for powdering in grinder-mixer.

87 gm. of the powdered leaves of *Murraya koenigii* was placed in a glass percolator (5-litr. capacity) with the addition of 1000 ml. of methanol so as to submerge the material satisfactorily. This was kept for 12 to 16 hrs (over night) at room temperature. Filtration of the extract through Whatman No.1 filter paper was carried out to collect the percolate. The process of extraction was repeated four times and the combined extract was evaporated to dryness under reduced pressure in a rotary evaporator keeping the temperature at 40°C (bath). The solid residual matter left was viscous in appearance and it was further dried by lypohilization. The yield was 14.84 gm. and the material was tested and found biologically active in both neutrophil or ex-vivo blood system.

Example – 2

The air-dried leaves of *Murraya koenigii* weighing 90 gms., were powdered and taken in a percolator. It was dipped in 800-ml chloroform. The submerged material was allowed to be extracted by the solvent for 12 to 16 hours (over night). The extract was filtered through Whatman No.1 filter paper and collected; this process was repeated for three times. The extract was evaporated to dryness in flash evaporator under reduced pressure at 40°C. The residual substance was then dried in a desiccator under high vacuum and the solid mass weighing 11.5gm was tested and found biologically active.

Example – 3

150 gm of fresh leaves of *Murraya koenigii* thoroughly washed in sterile water was homogenized with 750 ml of glass distilled water in a mixer-blender and then sonicated in an ultra sonic bath with 5 burst, each for 15min. Filtering through Whatman no.1 filter paper was carried out to separate the material extracted in water. This type of treatment for extraction was repeated for three times. The combined extract was lyophilized yielding a powdered material, 11gm in weight. This was then tested for biological activity.

Properties of the materials

The biologically active material obtained by examples 1 or 2 or 3 has the following properties:-

BNSDOCID: <WO 0232440A1 I >

1. The dried solid prepared as stated above was a dark colored material soluble in DMSO or dimethyl sulfoxide.

2. Thin layer chromatography analysis of the active material shows four spots having R_f values 0.60, 0.38, 0.24, and 0.15 respectively and R_f 0.73,0.60,0.34 and 0.14 in solvent system of chloroform and methanol taken in the ratio of 19:1.

3. The HPLC analysis of the active principles obtained from *Murraya koenigii* was carried out using Intersil ODS-3 (4.6x250mm) analytical column, with methanol as the solvent system and a flow rate 1.0ml /min. Detection at 254nm resolved the material into four peaks with the retention times 3.37, 3.49, 4.00, and 5.69 mins. respectively.

EVALUATION OF ANTIOXIDANT ACTIVITY IN AN IN VITRO SYSTEM

Biological evaluation was carried out as per the following procedure.

a) Inhibition of arachidonic acid oxidation in presence of the active material by neutrophil

Human peripheral blood neutrophil was prepared by standard method [Downey, G.P., Fukushima, T., Fialkow, L. and Waddel, T.K. (1995) Semin. Cell Biol. 6 345]. In brief, heparinised blood was treated with 2% gelatin (in normal saline) for half an hour to allow RBC separation at the bottom. The rest of the mixture was taken and subjected to gradient separation using Histopaque. The centrifugation was carried out at 1400 rpm at room temp. for 12 min. The neutrophil available as a separate layer at the bottom was collected and then made into suspension in sterile PBS or phosphate buffered saline.

(i) The neutrophil (1x104 /well of 24 well tissue culture plate) was treated with active material (20 g/ml) i.e. the extract obtained in Examples 1, 2 and 3 above, for 60 min., while the control did not contain any active material.

(ii) The neutrophil was primed with Phorbol Myristic Acetate (PMA) (10 M) for 30 min., followed by addition of active material (20 g/ml), or without it for 30 mins.

(iii) The neutrophil treated with active material (the extract) (20 g/ml) for 30 min. followed by PMA (10 M) activation for 30 min.

The oxygen consumption by the treated neutrophil was measured after ten minutes of the addition of arachidonic acid 10.2 µl (12.2 mg/ml in absolute alcohol) (Method. Enzymol. Ed. By Murphy, R.C, and Fitzpatrick F.A. Vol. 187 pp-268), in 2 ml volume of neutrophil suspension. The assay of the oxygen consumption was carried out by oxygen

sensor (of Hansatec with O_2 electrode control having Clark type probe). The results of inhibition of oxygen consumption due to arachidonic acid oxidation are set out inTable-1

Table–I

Inhibition of arachidonic acid oxidation by neutrophil

Treatment	Micromole oxygen consumed/10 min/	% of inhibition of O_2 consumption by active material with respect to	
		stimulation	without stimulation
A .PBS 60 min.	16.08	NA	---
B Active extract 60 min in '1'	12.85	----	20
C. PMA 30 min.	39.58	----	
D. Calcium ionophore 30 min.	64.01	----	
E. Active extract 30min + PMA 30 min.	21.42	46	
F. Active extract 30min.+Calcium ionosphere 30 min	25.3	60	

Calcium ionophore gave better results as there was about 60% inhibition while PMA effects 46% inhibition of O_2 consumption by active material present in *Murraya koenigii* leaf under in vitro neutrophil test. In both the stimulation remarkable inhibition of O_2 consumption indicates the efficacy of the material.

The results in Table-1 illustrated that the active factors in *Murraya koenigii* leaf acted as an inhibitor of oxygen consumption in presence of normal as well as activated human neutrophil and arachidonic acid. It suggests that oxidation of arachidonic acid is strongly inhibited by active factors present in *Murraya koenigii* leaves. The leukotrienes are the biological mediators of asthma and the oxidation of arachidonic acid is the rate-determining step for the synthesis of the leukotrienes. The strategy of asthma drug development is to search or synthesize compounds or prepare substances that inhibit the synthesis of leukotrienes or precisely cause the inhibition of the oxidation of arachidonic acid. The active material or compounds prepared according to the process of the invention,

not only inhibit the oxidation of arachidonic acid but are also free of toxicity and compatible for use in humans as the plant leaves are extensively used as food ingredients. Hence it qualifies as a drug for asthma.

b) Inhibition of arachidonic acid oxidation in presence of the active material by Human ex vivo Blood.

500µl of heparinised human whole blood was taken in each well of a 6-well micrometer plate to which the *Murraya koenigii* active material or none was added to have a final concentration of 50 gm/ml when the total volume was adjusted to 2 ml. with PBS. The plates were incubated for 2 hours with constant shaking. Next 10µl of arachidonic acid (12.2gm/ml in absolute alcohol stored under argon at –200C; Method. Enzymol. Ed. By Murphy, R. C, and Fitzpatrick F.A. vol. 187 pp-268) was added to each well for 10 mins. Prior to the addition of Phorbol Myristic Acetate (PMA, 10(M) or calcium ionophore A23187 (20 gm/ml), (Spaethe S.M, Snyder D.W, Pechous P.A, Clarke T, VanAlstyne E.L Biochem. Pharmacy. 43; 1992,377-82). After 30 mins of the addition of the stimulator, a YSI Clark oxygen probe containing oxygen-monitoring equipment monitored oxygen consumption.

Table –2

Inhibition of arachidonic acid in whole human ex vivo blood in presence of *Murraya koenigii* leaf material

Addition	Oxygen consumption (in nmol) /10 mins	Inhibition % of control
A. None N1(PMA treated)	39.99	-----
B. None N2 (Ca+ionophore)	62.41	----
C. None N3 (patient's blood)	32.15	-----
D. Whole leaf in N1 blood	22.1	45
E. Water extract in N1 blood	18.56	54
F. Methanol extract in N1 blood	10.70	73
G. Methanol extract in N2 blood	18.21	73

BNSDOCID: <WO_ _0232440A1_I_>

H. Methanol extract in N3 blood	11.70	70
Chloroform extract in N1 blood	11.11	72

* Patient having Eosinophil count 13 with 7 years of chronic asthma.

The leaf extract of *Murraya koenigii* was also found to be highly effective in the nitro-blue Tetrazolium reduction test indicating that the extract is a powerful antioxidant.

Again, the Nitro Blue Tetrazolium (NBT) reduction test was carried out as per the standard procedure [Rouiller,Y.B., and Mauel J.(1987) Infec.Immun. 55, 587]. Neutrophil was prepared and treated with or without PMA (30 min.) and followed by leaf extract of *Murraya koenigii* (60 min.) or without it. Then 100(l of NBT solution (3mg /ml) was added and cells were incubated for various intervals (0 to 120 mins). Formazan deposition was measured at 550 nm in an ELISA reader (Labsystem Multi Skan MS). The results showed that the activation of neutrophil with PMA was strongly inhibited about 6 times in the presence of the active material from the *Murraya koenigii* leaves at 120 min. The leaf preparation has strong antioxidant property which lend a firm support to recuperate from the condition that occurs during asthma.

EVALUATION OF *M. koenigii* FOR ASTHMA

The leaves of these plants are frequently used as flavor enhancer in South Indian cooked food. However, heating of the leaves denatures its anti-asthmatic property. Its use as a cooking item or in food recipe has been known for centuries and no untoward occurrence is yet evident indicating that all components of this leaf are totally safe for human consumption. Use of this plant especially in South Indian food never produced bowel discomfort or vomiting or stomach upset or any other problem. Especially it is neither bitter in taste nor odd in taste. After taking the leaf preparation no bowel discomfort or vomiting or stomach upset or any gastrointestinal problem was reported by any of the healthy volunteers. From all these information it is quite convincing that any preparation from this leaf as a drug for asthma will be absolutely safe.

The extract obtained from the plant *Murraya koenigii* exhibits properties that make it useful in the treatment of asthma. Accordingly, compositions comprising the extract, preferably, the lyophilized extract can be used for the treatment of asthma in humans. The extract, when dried appears as dark solids. Pharmaceutical compositions comprising the

extract of this plant along with additives, can be made in a variety of physical forms such as tablets, powders, syrups or other edible products. For the preparation of tablets, about 80-120mg of the extract from the plant is used. The tablets so prepared may be generally chewed or swallowed. The composition has no side effects and can be used without undue restriction. The composition was taken twice daily over a period of 30 days and found totally satisfactory without any complaints from any of the healthy volunteers.

An example of the composition prepared according to the invention is illustrated herein below.

LOCAL NAMES	PARTS SELECTED	AMOUNTS
M. paniculata Linn. Syn. M exotica (KAMINI)	Bark of root	80-100
H. abelmoschus LATAKASTURI	Seed	40-60
T.ammi JOWAN	Fruit	38-62
S. aromaticum LAVANGA	Dried flower buds	7-13
A.vasica Nees BASAK	Leaves	85-115
E. hirta PUSITOA	Whole plant	90-110
Murraya koenigii	Root bark and leaves	87-105

Below are representative examples of case studies wherein patients have been administered the composition of the invention. All these patients suffered from asthma, though the chronicity of the affliction varied from case to case.

Five volunteers (both female and male) were chosen with respect to their asthmatic condition depending on their sensitivity to the different causative agents that manifest their asthma.

CASE STUDIES:

Case-I

A female patient known for dust and cold sensitive severe asthma suffering for last seven years. Chest tightness, wheezing, cough, severe short-breath, night sleeplessness and

high mucus secretion during late night hours were the primary symptoms. She had to use inhaler twice or thrice daily.

For medication two spoonful of whole plant preparation were taken by her twice daily for fifteen days. The observed effects of this medication:

a) She did not have to use the inhaler except occasionally once in a day.

b) Wheezing and cough had totally stopped.

c) She was completely free from chest tightness.

d) She appeared physically normal and capable of strenuous activities like walking climbing up staircase to cross floors upto third level.

Case – II

Two volunteer patients (one male and another female) with genetic predisposition for asthma suffering from sleeplessness during night time, very prone to asthmatic condition resulting from sensitively to pollution and shortness of breath they needed to take inhaler when there was a severe night attack. They followed the medication as in case - 1 for one month.

i) Uptill the ninth month of observation they did not have any asthmatic attack,

ii) No shortness of breath was reported by them,

iii) In case of the male patient smoking was a risk enhancer for frequent episodes of asthmatic attack. But he did not have any breathing discomfort even if he smoked more than usual occasionally.

iv) Earlier the female patient had shortness of breath while taking long walks or climbing stair cases only upto a single floor but after the treatment she did not have any trouble as described above breathing even when she climbed upto third level.

Case - III

Another young volunteer patient who suffers from periodical severe attack with acute shortness of breath and often had to be taken to hospital for fresh oxygen supply to ease out breathing acuteness.

During one such episode of acute attack of shortness of breath, he took the leaf preparation and the effects as observed were:

i) He did not have to go to the hospital,

ii) Since then he took this medication for a month, and

iii) His every day activities have increased remarkably and till date there has not been any occurrence of highly acute respiratory distress.

Case-IV

An aged woman patient suffering from sleeplessness during night and extreme shortness of breath and showing no response to the modern medication including inhaler.

She was administered the leaf preparation which had to be taken twice daily for fifteen day and her condition has improved remarkably.

Thus, the application herein provides a simple fast and inexpensive process for preparation of a composition from an abundantly available plant for relief, treatment and cure of asthmatic problem satisfactorily. The method of administration of the plant material is also simple. The success rate is 100% among the volunteers. There has been no case of relapse during the observation time of 6 months. No adverse or side effects were observed after the treatment.

The major advantages of the present invention are:

1. It is expected to provide relief, treatment and cure of asthmatic problem satisfactorily.
2. It is prepared from a plant material, which has been used in food for centuries and is biocompatible and non-toxic.
3. The plant is abundantly available.
4. Method of preparation of the active material /principle is simple, fast and inexpensive.
5. Its cultivation and propagation is easy as it grows an all kinds of soil.

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CLAIMS:

1. Pharmaceutical composition useful in the treatment of asthma, said composition comprising an effective amount of the extract obtained from the plant *Murraya koenigii* together with, or optionally associated with a pharmaceutically acceptable additive.

2. A composition as claimed in claim 1 wherein the additives comprise powder or extracts of plants selected from *M. paniculata Linn*, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica Nees*, *E.hirta*, and *Murraya koenigii*.

3. A composition as claimed in claim 1 wherein the additives are present in the range of 80-100 mg of *M. paniculata Linn*, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T. ammi*, 7-13 mg of *S. aromaticum*, 85 - 115 mg of *A.vasica Nees* and 90-110 mg of *E.hirta*.

4. A composition as claimed in claim 1 comprising :

M. paniculata Linn. Syn. M exotica (KAMINI)	90mg
H. abelmoschus (JOWAN)	50mg
T. ammi (LAVANGA)	50mg
S. aromaticum (BASAK)	10mg
A.vasica Nees (PUSITOA)	100mg
E.hirta	100mg
Murraya koenogii (Suravi Neem)	100mg

5. A composition as claimed in claim 1 wherein the extract of the plant *Murraya koenigii* is present in the range of 87-105 mg per dose.

6. A composition as claimed in claim 1 wherein the additives are preferably present in an amount 90 mg of *M. paniculata Linn*, 50 mg of *H. abelmoschus*, 50 mg of *T. ammi*, 10 mg of *S. aromaticum*, 100 mg of *A.vasica Nees*, 100 mg of *E. hirta*, and 100 mg of *Murraya koenigii*. per dose.

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7. A composition as claimed in claim 1 wherein the extract of the plant *Murraya koenigii* comprises active principles which are dark colored solids, soluble in dimethylsulfoxide.

8. A process for preparing an extract from the plant *Murraya koenigii*, useful in the treatment of asthma, said process comprising the steps of pulverising plant materials obtained from plant *Murraya koenigii*, extracting the plant material with a solvent at ambient temperature, concentrating the extract by filtering and evaporating it under reduced pressure and lyophilizing the concentrate to obtain a lyophilized extract containing active principles of the plant *Murraya koenigii*.

9. A process as claimed in claim 8 wherein the plant materials are obtained from plant parts of *Murraya koenigii* selected from garden fresh leaves or leaves dried under shade.

10. A process as claimed in claim 8 wherein the leaves are pulverized by conventional methods to get homogenized leaves.

11. A process as claimed in claim 8 wherein the plant materials are extracted with solvents selected from hydrocarbon solvents, chlorinated solvents, etheral solvents, ketonic solvents, alcohols, water and buffers.

12. A process as claimed in claim 11 wherein the solvents are selected from the group consisting of petroleum ether (BP 40 – 60°C), petroleum ether (60°C - 80°C), benzene, pentane, hexane, chloroform, dichloromethane, carbon tetrachloride, diethyl ether, tetrahydrofuran, dioxane, acetone, cyclopentanone, ethyl acetate, ethyl formate, methanol, ethanol, n-butanol, water and buffers.

13. A process as claimed in claim 8 wherein the concentration of the extract is effected by filtering and evaporating the solvents under reduced pressure and at a temperature in the range of 20°C – 80°C.

14. A process as claimed in claim 8 where in the extract obtained from the plant *Murraya koenigii* comprises active principles which are dark colored solids soluble in dimethylsulfoxide.

15. A process as claimed in claim 8 wherein the active principles obtained from the plant *Murraya koenigii* are biocompatible and non-toxic in nature.

16. A process as claimed in claim 8 wherein the extract when subject to chromatography exhibits active principles having R_f values 0.73, 0.60, 0.34 and 0.14 respectively in chloroform.

17. A process as claimed in claim 8 wherein the extract when subject to chromatography with methanol and chloroform in the ratio 19:1, exhibits R_f values 0.60, 0.38, 0.24 and 0.15 respectively.

18. A process as claimed in claim 1 wherein the active principles exhibit four peak with retention times 3.37, 3.49, 4.0 and 5.69 respectively in methanol as solvent at 254nm.

19. A process as claimed in claim 8 wherein the extraction process is carried out for a period ranging from 1 – 120 hrs, preferably between 12 – 16 hrs.

20. Use of the extract obtained from the plant *Murraya koenigii* for the manufacture of a pharmaceutical composition useful in the treatment of asthma.

21. Use as claimed in claim 20 wherein the pharmaceutical composition comprises lyophilized extract obtained from *Murraya koenigii* together with other conventional additives.

22. Use as claimed in claim 20 wherein the composition is administered orally for treatment of mild or acute asthma.

23. Use as claimed in claim 20 wherein the dosage level of the composition is in between 325-600 mg twice daily for a period ranging from 3 to 30 days.

24. Use as claimed in claim 20 wherein the dosage level is in between 325-600 mg twice daily for the period ranging from 3 to 15 days for mild asthmatic condition, and 15 – 30 days for acute asthmatic condition.

25. Use as claimed in claim 20, wherein the additives are selected from *M. paniculata Linn, H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees, E.hirta,* and *Murraya koenigii.*

26. Use as claimed in claim 20, where in the additives are present in a range of 80-100 mg of *M. paniculata Linn,* 40-60 mg of *H. abelmoschus,* 38-62 mg of *T. ammi,* 7-13 mg of *S. aromaticum,* 85-115 mg of *A.vasica Nees,* 90-110 mg of *E. hirta,* and 87-105 mg of *Murraya koenigii* per dose.

27. Use as claimed in claim 20, wherein the additives are preferably present in an amount 90 mg of *M. paniculata Linn,* 50 mg of *H. abelmoschus,* 50 mg of *T. ammi,* 10 mg of *S. aromaticum,* 100 mg of *A.vasica Nees,* 100 mg of *E. hirta,* and 100 mg of *Murraya koenigii* per dose.

28. Use as claimed in claim 20, wherein the additives *M. paniculata Linn, H. abelmoschus, T. ammi, S. aromaticum, A. vasica Nees, E. hirta,* and *Murraya*

koenigii are administered to include properties such as antidiahorial, antiseptic, carminative, stimulation, anti-cough, anti- bronchitis and nourishment.

29. Use as claimed in claim 20, wherein the additives are obtained from : *M. paniculata Linn* (bark or root), *H. abelmoschus* from dried flower buds, *T. ammi* from leaves, *S. aromaticum* from whole plant *A.vasica Nees* from root, *E. hirta* from bark, and *Murraya koenigii* from leaves.

30. An anti-oxidant composition for human beings and animals, said composition comprising a effective amount of extract obtained from the plant *Murraya koenigii* together with or optionally associated with additives.

31. A composition as claimed in claim 30 wherein additives comprise powder or extracts of plants selected from *M. paniculata Linn, H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees, E. hirta,* and *Murraya koenigii.*

32. A composition as claimed in claim 30, wherein the additives are selected from *M. paniculata Linn, H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees, E. hirta,* and *Murraya koenigii* in the form of bark or root; seed; fruit; dried flower buds; leaves; whole plant; and root, bark, leaves, respectively.

33. An anti-asthma agent obtained from the plant *Murraya koenigii.*

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AMENDED CLAIMS

[received by the International Bureau on 15 February 2002 (15.02.02);
original claims 1-33 replaced by new claims 1-28 (3 pages)]

CLAIMS

1. A synergistic pharmaceutical composition useful for the treatment of asthma, said composition comprising an effective amount of extract obtained from the plant *Murraya koenigii* together with additives selected from plant parts of *M. paniculata Linn*, *H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees* and *E.hirta* and optionally with pharmaceutically acceptable additives.
2. The composition as claimed in claim 1 wherein the additives are selected from powdered plant parts or lyophilized extracts of plants from *M. paniculata Linn*, *H. abelmoschus, T. ammi, S. aromaticum, A.vasica Nees* and *E.hirta.*
3. The composition as claimed in claim 1 wherein the additives are present in the range of 16 – 20 % by wt of *M. paniculata Linn*, 8 – 12 % by wt, of *H. abelmoschus*, 7.6- 12.4 % by wt of *T. ammi,* 1.4 – 2.7% by weight, of *S. aromaticum*, 17-23 % by wt of *A.vasica Nees* and 18-22 % by wt of *E.hirta.*
4. The composition as claimed in claim 3, wherein the additives are present in the range of 17.7 – 19 % by wt of *M. paniculata Linn*, 9.3 – 10.6 % by wt, of *H. abelmoschus*, 8.9- 10.9 % by wt of *T. ammi,* 1.6 – 2.7% by weight, of *S. aromaticum*, 20.0–20.3 % by wt of *A.vasica Nees* and 19-21 % by wt of *E.hirta.*
5. The composition as claimed in claim 4, wherein said composition comprising 20 % by wt of *Murraya koenigii* along with 18 % by wt of *M. paniculata Linn*, 10 % by wt of *H. abelmoschus*, 10 % by wt of *T. ammi,* 2 % by wt of *S. aromaticum*, 20 % of *A.vasica Nees*, 20 5 by wt of *E. hirta.*
6. The composition as claimed in claim 1 wherein the leaf extract of the plant *Murraya koenigii* is present in the range of 17.4 to 21 %, preferably 19-20 % by weight.
7. The composition as claimed in claim 1 wherein the extract of *Murraya koenigii* is obtained from garden fresh leaves or shade dried leaves.
8. The composition as claimed in claim 1 wherein the extract of the plant *Murraya koenigii* comprises active principles, which are dark colored solids soluble in dimethylsulfoxide.
9. The composition as claimed in claim 1, wherein the active principles obtained from the plant *Murraya koenigii* are biocompatible and non-toxic in nature
10. A process as claimed in claim 1, wherein the extract obtained from the plant *Murraya koenigi* when subjected to chromatography with chloroform and

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methanol in the ratio 1:19, exhibits R_f values 0.60, 0.38, 0.24 and 0.15 respectively.

11. The composition as claimed in claim 1, wherein the additives are obtained from bark or roots of *M. paniculata Linn*, seeds of *H. abelmoschus,* fruits of *T. ammi*, dried flower buds of *S. aromaticum*, leaves of *A.vasica Nees*, whole plant of *E. hirta* and leaves of *Murraya koenigii.*

12. A composition as claimed in claim 1, wherein said composition is administered orally for the treatment of mild to acute asthma.

13. A composition as claimed in claim 1, wherein said composition is administered at a dosage of 325-600 mg powder or 20 ml of liquid preparation, at least thrice a day for a period ranging from 30 to 120 days.

14. A composition as claimed in claim 1, wherein said composition is administered for a period ranging from 30 to 45 days for mild asthmatic condition and from 45 – 120 days for acute asthmatic condition.

15. A process for preparing lyophilized extract from the plant *Murraya koenigii*, useful in the treatment of asthma, said process comprising the steps of pulverizing plant materials obtained from plant *Murraya koenigii*; extracting the plant material with a solvent at an ambient temperature, concentrating the extract by filtering and evaporating it under reduced pressure and then lyophilizing the concentrate to obtain a lyophilized extract of the plant *Murraya koenigii.*

16. A process as claimed in claim 15 wherein the plant materials are obtained from leaves of *Murraya koenigii* selected from garden fresh leaves or leaves dried under shade.

17. A process as claimed in claim 15 wherein the leaves are pulverized by conventional methods to get homogenized leaves.

18. A process as claimed in claim 15 wherein the plant materials are extracted with solvents selected from hydrocarbon solvents, chlorinated solvents, etheral solvents, ketonic solvents, alcohols, water and buffers.

19. A process as claimed in claim 15 wherein the solvents are selected from the group consisting of petroleum ether (BP 40 – 60°C), petroleum ether (60°C - 80°C), benzene, pentane, hexane, chloroform, dichloromethane, carbon tetrachloride, diethyl ether, tetrahydrofuran, dioxane, acetone, cyclopentanone, ethyl acetate, ethyl formate, methanol, ethanol, n-butanol, water and buffers.

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20. A process as claimed in claim 15 wherein the concentration of the extract is effected by filtering and evaporating the solvents under reduced pressure and at a temperature in the range of 20°C – 80°C.

21. The composition as claimed in claim 15, wherein the extract of the plant *Murraya koenigii* comprises active principles, which is a dark colored solid soluble in dimethylsulfoxide.

22. A process as claimed in claim 15 wherein the active principles obtained from the plant *Murraya koenigii* are biocompatible and non-toxic in nature.

23. A process as claimed in claim 15 wherein the extract when subjected to chromatography exhibits active principles having R_f values 0.73, 0.60, 0.34 and 0.14 respectively in a solvent system comprising chloroform and methanol in the ratio 1:19.

24. A process as claimed in claim 15 wherein the extract when subjected to chromatography with chloroform and methanol in the ratio 1:19, exhibits R_f values 0.60, 0.38, 0.24 and 0.15 respectively.

25. A process as claimed in claim 15 wherein the active principles exhibit four peaks with retention times 3.37, 3.49, 4.0 and 5.69 minutes respectively in methanol as solvent at 254nm.

26. A process as claimed in claim 15 wherein the extraction process is carried out for a period ranging from 1 – 120 hours, preferably between 12 – 16 hrs.

27. A process as claimed in claim 15 wherein, the extract obtained from the leaves of the plant *Murraya koenigii* is used for the manufacture of a pharmaceutical composition useful in the treatment of asthma.

28. A process as claimed in claim 15 wherein, the said extract extract obtained from the leaves of the plant *Murraya koenigii* is mixed with other conventional additives to obtain pharmaceutical composition.

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STATEMENT UNDER ARTICLE 19(1)

The claims have been amended in order to achieve clarity without expanding the scope of the invention. No new matters have been added.

INTERNATIONAL SEARCH REPORT

national Application No
PCT/IN 00/00102

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/78 A61P11/06 //(A61K35/78,35:78)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, BIOSIS, EPO-Internal, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	SINGH Y N: "Traditional medicine in Fiji: some herbal folk cures used by Fiji indians" J ETHNOPHARMACOL, vol. 15, no. 1, 1986, pages 57-88, XP001000897 * Table 1, page 81, No. 68 * * p. 68, No. 22 : Euphorbia hirta * * p. 77, No. 54 : Syzigium aromaticum * * p. 84, No. 80 : Trachyspermum ammi * --- -/--	1,20,30, 33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
25 June 2001	29/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Laffargue-Haak, T

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BNSDOCID: <WO__0232440A1_1_>

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 01, 31 January 1997 (1997-01-31) & JP 08 225420 A (INAHATA KORYO KK;NIPPON FUNMATSU YAKUHIN KK), 3 September 1996 (1996-09-03) abstract	1,7-19, 30-32
X	PATENT ABSTRACTS OF JAPAN vol. 1995, no. 08, 29 September 1995 (1995-09-29) & JP 07 138126 A (MIKIMOTO PHARMACEUT CO LTD;OTHERS: 01), 30 May 1995 (1995-05-30) abstract	1,7-19, 30-32
X	KHAN BEENA A ET AL: "Anti-oxidant effects of curry leaf, Murraya koenigii and mustard seeds, Brassica juncea in rats fed with high fat diet." INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY, vol. 35, no. 2, 1997, pages 148-150, XP001000038 ISSN: 0019-5189 cited in the application tables 1-3	1,7-19, 30

INTERNATIONAL SEARCH REPORT

Information on patent family members

National Application No
PCT/IN 00/00102

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 08225420 A	03-09-1996	NONE	
JP 07138126 A	30-05-1995	NONE	

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